MILK QUALITY OF DAIRY GOAT BY GIVING FEED SUPPLEMENT AS ANTIOXIDANT SOURCE

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ABSTRACT

Free radical levels can be higher than the level of endogenous antioxidants in the body so that uncomfortable conditions in the body of dairy goats could happen. To anticipate this uncomfortable conditions will be given feed supplement (FS) as source of antioxidants (AOX). FS contain mixture pineapple rind meal and antioxidant minerals (AOXM) each 25 ppm Zn and 10 ppm Cu. This experiment was carried out to investigate the effect of feed supplements as antioxidant source on milk quality of dairy goats. Sixteen Etawah dairy goats in the second lactation were used in the experiment that conducted using randomized block design with 4 treatments and 4 replicates. The treatments were R0 (grass + concentrate), R1 (R0 + FS containing 0.04 % AOX), R2 (R0 + FS containing 0.06% AOX), R3 (R0 + FS containing 0.08 % AOX). The data collected were analyzed using Anova. The result of phytochemicals analysis indicated that feed supplement contained flavonoid, polyphenols, sesquiterpen, mopnoterpen, steroids, quinones and saponins. The results of study showed that there were difference ($p<0.05$) among treatments on blood and milk cholesterol and milk lactose, but there were no difference ($P>0.05$) on milk yield, milk fat, milk protein and milk antioxidant. The conclusion of this study was the feed supplements containing 0.08 AOX produced the best response to milk quality of dairy goats.

Keywords: dairy goats, feed supplement, antioxidants

INTRODUCTION

In the body there is a natural balance between the formation of free radicals during the normal metabolism of the cells and the endogenous antioxidant capacity of the animal that would prevent free radicals from accumulating and harming the cells. However, the level of free radicals can exceed the antioxidant capacity of the animal leading to oxidative stress (Weiss, 1998). High producing dairy animal are prone to oxidative stress, and the situation can be exacerbated under certain environmental, physiological, and dietary conditions (Castillo *et al*., 2005).

Physiological conditions affect the productivity of animals. Free radical levels can be higher than the endogenous AOX and resulting uncomfortable condition. This condition will give negative effect to the productivity of dairy goats especially on milk yield and milk quality. To anticipate this condition it is advised to give feed that contain sources of AOX. Bellville-Nabet (1996) reported that natural AOX found in many kinds of food can be categorized into two groups, the first group of nutrients consisting of vitamins (A, B, C, E) and minerals (Zn, Cu, Se), the second group of non-nutritional food consisting of phenol compounds, flavonoids, steroids, alkaloids, terpenoids, tannins and saponins.

Oxidative stress in a living organism is a result of an imbalance between reactive oxygen metabolites (ROM) production and neutralizing capacity of antioxidant mechanisms (Sies, 1991). Oxidative stress can lead to the modification of important physiological and metabolic functions. Miller and Brezeinska-Slebodizinska (1993) reported that oxidative stress could alter the physiology and could cause pathologies.

Use of antioxidant (AOX) may be an alternative to improve lipid metabolism and oxidative status. AOX was reported to be effective to improve oxidative balance and performance in lactating cows by improving rumen metabolism (Vázquez-Añón *et al*., 2008). Dietary AOX did not affect on milk protein, fat, lactose, total solids and non-fat solids of cows milk (Wang *et al*., 2010).

Antioxidants are naturally or synthetic chemicals in foods that help to counter the detrimental effects of reactive oxygen species (ROS), free radicals and causes degenerative human diseases such as cancer, heart diseases and cerebrovascular diseases (Wresburger, 2002). Natural antioxidant present in foods, especially fruit and vegetables consisting three main groups, vitamins, phenolics and carotenoids. Ascorbic acid and phenolic antioxidants are known as hidrolifik, whereas the lipophilic antioxidants known as carotenoids (Halliwell *et al*., 1996).

Antioxidants present in food or as supplements such as vitamin E, vitamin C, ß-carotene, flavonoids could improve health protection against free radicals as an attempt to prevent damage caused by oxidation process. It encourages more exploration of natural materials as source of antioxidants. Howard *et al*. (2002) reported that sources of antioxidants could be found in many vegetables, fruits, herbs and spices. Antioxidant activity found in flavonoids and polyphenols (Panovskai *et al*., 2005; Huda-Faujan *et al.*, 2009), together with vitamin C and carotenoids protect the tissue from oxidative stress (Scalbert and Williamson, 2000).

Pineapple (*Ananas comosus* L. Merr) is the fruit containing high vitamin C and natural antioxidants that could inhibit the development of clinical conditions including heart disease and cancer in humans (Diplock *et al*., 1994; Mahdavi *et al*., 2010). It also contains phenolic compounds and β-carotene (Gardner *et al*., 2000; Charoensiri *et al*., 2009; Kongsuwan *et al*., 2009). Pineapple is consumed not only as fresh fruit but also material for processing such as pineapple jam, sweets, syrups, and others. Winarsi (2007) reported that the rind of fruit contained high antioxidants than the pulp so that it also has the potential to contain antioxidants.

The study was conducted to determine of pineapple rind meal mixed AOX mineral Zn and Cu as feed supplement so it is expected to improve milk quality of Etawah dairy goats.

MATERIALS AND METHODS

Animal and diets

Sixteen Etawah dairy goats of second lactation were used in this experiment. Goats were housed in the individual stall barn, producing 0.467 liters milk/day.

Pineapple rind was dried in the oven at 40-50ºC for 2 days and then was ground. It was then mixed with antioxidant minerals (AOXM) each 25 ppm Zn and 10 ppm Cu. Mixture Pineapple rind meal (PSM) and AOXM called feed supplement (FS). FS was taken for phytochemical analysis and look at the content nutrient and non nutrient antioxidant

There were four dietary treatments used in this study, namely 1) basal diet (control) (BD) consisting of grass (70%) and commercial concentrate (30%) 2). BD + FS containing 0.04% AOX, 3) BD + FS contain 0.06% AOX, 4) BD + FS containing 0.08% AOX. Each of the diets was assigned to the goat in randomized block design with 4 treatments and 4 replications.

Goats were fed twice daily in amounts adequate to allow maximum access to feed at the day and to ensure 10% body weight gain. The composition of the treatment diet is shown in Table 1.

Goats were milked every day at 07.00 and milk samples were taken on d 0, 30 and 60 days of experiment, stored in freezer −20°C until analyzed for antioxidant, cholesterol, protein, lipid and lactose content (Table 1).

Data colection

Observed variables were the phytochemical content of pineapple rind powder analyzed in the laboratory of the Faculty of Pharmacy, Padjadjaran University-Bandung. Milk yield as long as lactation was measured everyday starting fourth day after parturition. Milk quality included milk protein was analyzed using Hadiwiyoto method (1982), milk fat was analyzed with Gerber method according Sudono *et al*. (1999) and milk lactose analyzed with calorimetry method according Apriyantono *et al.* (1989). The antioxidant and cholesterol content of milk were analyzed at Laboratory Center for Agricultural Postharvest Research and Development, Ministry of Agricultutre, Bogor.

Data Analysis

Data were subjected to one-way ANOVA using SAS program (1988). Difference among treatment means ($P<0.05$) was distinguished using Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Bioactive Component of Feed Supplement

Some polyphenols from plants have the

ability as an antioxidant that protects cells from oxidative damage (Young *et al.*, 2003) by neutralizing reactive oxidants (Ahmed and Beigh, 2009). Flavonoids have antioxidant capacity (Beecher, 2003; Zang and Hamauzu, 2003).

Pineapple rind meal contains flavonoids 3.47%. Flavonoids have antioxidant capacity (Beecher, 2003; Kondakova *et al.*, 2009). Flavonoid compounds to flavonols group consists of quercetin, mirisetin and kaemperol (Lin *et al.*, 2006). Quercetin has high antioxidant activity (Moskaug *et al*., 2005) by chelate iron ions, inhibiting lipid peroxidation, capturing free radicals and oxygen active (Kuresh *et al.,* 2004). Antioxidant activity of flavonoids is not only through its structure, but also its presence in the membrane. Protective effect of flavonoids is important for application in diseases caused by free radicals (Saija *et al.*, 1995).

Pineapple rind meal also contains vitamin C and beta carotene (Table 2). Flavonoids content was highest than vitamins and beta carotene. Vitamin C was known as an antioxidant (Kondakova *et al.*, 2009) because of its ability to donate electrons (Padayatty *et al*., 2003) which can prevent cell damage from oxidation of fat (Evans, 2000). Beta carotene has antioxidant activity (Kiokias and Gordon, 2003) and protects cells from oxidative stress induced by Fe (Matos *et al.*, 2006) As an antioxidant, vitamin C works as an electron donor by moving one electron to the Cu metal compound (Padayatty *et al.*, 2003) Vitamin C is an essential phytonutrients for the metabolism of living cells that occurs in different concentrations in natural foods especially fruits and their products. It is considered as the major antioxidant in the diet. Gardner *et al*. (2000) have revealed that Vitamin C accounted for 65-100% of antioxidant capacity of citrus juices.

Bioactive compounds and antioxidant capacities of pineapple are vitamin C contents that were 18.88 mg/100 g FW, β-carotene contents were found to be relatively low in and total phenolic contents were 26.20 mg GAE/ 100 FW (Kongsuwan *et al*., 2009). The amounts of these bioactive compounds have been widely reported in pineapple varieties with differences in their concentration. Differences in the concentration may be due to a number of factors, including cultivars, natural variation of fruit, climatic condewitions or soil, fertilizer or geographical origin (Charoensiri *et al*., 2009). Vitamin C is a powerful antioxidant found in many fruits. A strong positive relationship between the amount of vitamin C, but not of total phenolics and bioflavonoids, and total antioxidant activity has been reported (Kim *et al.*, 2002).

Cholesterol Content in Blood of **Dairy Goats**

The results showed that blood cholesterols, HDL, LDL and triglycerides of dairy goats was affected by FS ($P<0.05$) as shown in Table 3. Treatment of BD plus FS was higher than control. Phytochemical analysis showed that rind of pineapple meal containing bioactive component such as phenolic and non phenolic compounds that functioned as antioxidants can prevent and increase cholesterol and LDL. Naidu and Thippeswamy (2002) reported that -phenolic and non phenolic compounds have function as AOX and preventing oxidation of LDL. Winarsi (2007) also stated that phenols have cardioprotective effect as powerful antioxidant. Phenolic compounds can prevent LDL oxidation 20 times stronger than vitamin E. The results of analysis showed that the treatment was significantly ($P <0.05$) to all variables. Decrease of cholesterol, LDL and triglycerides and increase of HDL occurred in BD+0.08 AOX treatment as bioactive components contained in pineapple rind meal was higher than other treatments. Kinsella *et al.* (1993) reported that plavonoid contained in food plants can be functioned as an antioxidant. This relates to the ability to capture free radicals and radical peroxysalts so that it will be effective in inhibiting lipid oxidation, especially can lower cholesterol. In addition, Zn can be stored in the form of metallotionin which is the eater of free radicals. Metallotionin is an intracellular protein which has tight binding with Zn and Cu (King, 2000).

Engle *et al.* (2001) reported that giving of 10-20 mg Cu/kg DM can decrease serum cholesterol and fat Angus cattle. Solaiman *et al*. (2006) stated that Cu supplementation of 100 mg / day changed the serum lipid profile, decreased carcass fat of goat kids fed by high-concentrate. Decrease carcass fat has health benefits for humans. Engel *et al.* (2001) gave the reason that Cu stimulates the synthesis of cholesterol in the liver. Cholesterol metabolism is also influenced by genetics and gender. Plasma cholesterol was also influenced by a complex interaction of metabolism in the body such as the absorption by the intestine, the synthesis and metabolism in the liver, and peripheral blood vessels.

Pineapple rind meal contains saponins of 5.29%. Several studies showed that saponins

Table 1. Nutrient Content in Dietary Treatments

Nutrient	Diet			
	BD (Control)	BD + 0.4% AOX	BD + 0.6% AOX	BD + 0.8% AOX
Dry Matter (%)*	87.93	88.06	88.12	88.18
Crude Protein (%)*	10.58	11.13	11.40	11.67
Ether Extract (%)*	2.94	3.13	3.23	3.33
Crude Fiber (%)*	21.96	23.15	23.73	24.31
Ash*	10.46	10.93	11.17	11.41
Nitrogen Free Extract*	41.99	50.95	55.42	59.89
Zn (ppm) **	10.38	36.36	36.87	37.36
Cu (ppm) **	1.35	11.49	11.56	11.63
Ca*	0.61	0.66	0.69	0.72
Total Phosfor*	0.50	0.52	0.53	0.54
NDF*	61.89	66.02	68.08	70.14
ADF*	29.64	31.14	31.89	32.64
Hemicellulose*	1.85	2.08	3.93	5.24
Cellulose*	19.82	21.77	21.77	22.41
Lignin*	6.63	7.02	7.22	7.42

*) Results of analysis at Laboratory of Nutrients and Animal Feed, Bogor Agicutural University
**) Results of analysis at Center for Agricultural Postharvest Research and Development, Ministry of Agriculture, Bogor

Table 2. Bioactive Components of Feed Supplement

Bioactive Component	Content
Antioxidant Total (mg/100g)	38.95
Vitamins C (mg/100g)	24.40
ß- caroten (ppm)	59.98
Phenolic (ppm)	32.69
Galat acid (mg/g)	5.67
Flavonoid (%)	3.47
Quercetin (%)	1.48
Saponin (%)	5.29

compound from various sources can lower serum cholesterol levels in animals and humans. Matsui *et al.* (2009) reported that saponins of tea leaf extracts have antihiperkolesterolemia activity by 72% and the addition of 0.5% saponin on high cholesterol feed can inhibit the increasing serum cholesterol levels. Saponins also stimulate the reduction of cholesterol and triglycerides in the liver and increases cholesterol excretion in feces. This indicates that saponins can inhibit the absorption of cholesterol in intestine.

Milk Yield and Milk Composition of Dairy Goats

The yield and composition of goat milk very widely and this variation is attributed to breed, parity, stage of lactation (Pal *et al.*, 1996), age, geographical location, season, diet, health and management of goats (Sing and Sengar, 1990). Breeds like Alpine and Saanen produce milk with lower fat, protein and casein contents mainly because of higher milk yield in these breeds (Tziboula-Clarke, 2003). Similarly with the progress of lactation period, fat, ash and total solids are reported to increase while lactose content decrease (Pal *et al.*, 1996).

The results showed that the antioxidant content of milk was not affected by FS but the treatment showed significant effect (P <0.05) on cholesterol Table 4). Cholesterol content of dairy milk is the lowest in FS containing 0.08% AOX. FS contain bioactive component such as flavonoids and vitamins C.

Dietary antioxidant has no effect on milk

Table 3. Effects of Feed Supplements Containing Antioxidants on Total Cholesterol, LDL, HDL and Trygliceride of Dairy Goats Blood

Parameter	Diet				SEM	P-Value
	BD (control)	BD+0.04% AOX	BD+0.06% AOX	BD+0.08% AOX		
Kolesterol total (mg/dL)	138.45[a]	133.46[a]	137.65[a]	121.99[b]	1.105	0.0030
HDL (mg/dL)	43.22[a]	40.67[a]	42.62[a]	37.95[b]	0.743	0.0160
LDL (mg/dL)	60.68[a]	82.47[b]	86.95[b]	83.43[b]	0.328	0.0290
Trygliceride (mg/dL)	132.73[a]	128.52[b]	126.60[b]	116.47[c]	2.432	0.0367

Different superscripts within the same row indicate significantly different (P <0.05).
BD = Basal Diet
AOX = Antioxidant

Table 4. Effects of Feed Supplements Contain Antioxidants on Milk Yield, Total Antioxidant, Cholesterol, Protein, Fat and Lactose of Dairy Goats Milk

Parameter	Diet				SEM	P-Value
	BD (control)	BD+0.04% AOX	BD+0.06% AOX	BD+0.08% AOX		
Milk yield (l/d)	0.44	0.49	0.59	0.57	2.41	0.1340
Antioxidant (mg/100ml)	30.968	31.263	33.670	33.893	3.606	0.1563
Cholesterol (mg%)	4.209[a]	4.178[a]	3.664[b]	3.519[b]	0.090	0.0199
Protein (%)	3.427	3.433	3.772	3.783	0.606	0.2028
Fat (%)	6.292	6.422	6.457	6.725	0.095	0.3088
Lactose (%)	4.584[a]	4.796[a]	5.229[b]	5.616[b]	0.172	0.0272

Different superscripts within the same row indicate significantly different (P <0.05).
BD = Basal Diet
AOX = Antioxidant

protein, fat, lactose, total solids and non-fat solids of cows milk (Wang *et al.*, 2010). Aoki *et al.* (2010) stated that trehalose supplementation in the diets of dairy cows produced milk with low lipid peroxide and high AOX content, suggesting that trehalose could be useful as a supplement for inhibiting oxidative stress, enabling the production of low-lipidperoxide and high-AOX milk for human consumption and decreasing the unwanted effects of lipid oxidative odor in cow's milk. According to Haenlein (2002) that genetic influences on nutritional composition of milk and it has a heritability value of 50%. In other words, 50% of high and low nutrient composition of milk is determined by dietary factors and management. Bruhn (2006) explained that the type of feed affects nutrient composition of milk produced by cattle and feed quality will affect the metabolism in the body of livestock that will affect the availability of energy and nutrients for the synthesis of milk components.

The results of this study showed that FS containing AOX influenced ($P<0.05$) milk lactose but not significantly ($P>0.05$) affect milk yield, fat and protein content of milk Table 4). Highest levels of milk lactose was in the BD + 0.08% AOX treatment. This shows that the active component such as plavonoid and vitamin C can protect fatty acids from oxidation (Zielinska *et al.*, 2001), and these compounds have the ability to reduce free radical formation and capture the free radicals that can cause degradation of fatty acids with the protection of fatty acids from oxidation (Pazos *et al.*, 2005; Juntachote *et al.*, 2007)

Vazquez-Anon *et al*. (2008) reported that feeding AOX improved lactation performance by the increasing of DMI, FCM, and milk fat yield independent of the level of oxidation of the fat, and by the increasing of the plasma antioxidant enzymes and status of the cow. Changes in milk fat composition indicated decreased oxidation and ruminal biohydrogenation. Feeding oxidized fat decreased DMI without negatively affecting milk yield but at the expenses of antioxidant status of the cow. The negative effect of feeding oxidized fat was partially ameliorated with AOX supplementation

CONCLUSION

Feed supplement containing AOX of pineapple rind meal with Zn and Cu supplementation would reduce blood and milk cholesterol and increase milk lactose, but has no effect on antioxidant, protein and fat of dairy goats milk.

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